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13. ABSTRACT ***(Maximum 200 Words)***

We have developed a unique magnetic resonance imaging multi voxel pulse sequence producing spectroscopic images of key metabolites found in breast cancer, and validated our work with *in vitro* spectra and pathology. We have shown that choline peaks are often present in breast cancer, and that our MRS sequence was unaffected by intravenous contrast, increasing its clinical utility as an adjunctive study to clinical breast MRI scans. However, MRS may have a major limitation in that specific tumor histologies that have dispersed cells, such as invasive carcinomas with some invasive lobular carcinoma features may *not* show choline peaks. Technical developments to date have significantly contributed towards the goal of making MR spectroscopic imaging a clinically useful procedure that could be implemented at the time of a contrast-enhanced MRI scan. Important applications for this technique include distinction of a breast cancer recurrence from a post-biopsy scar within 24 months of initial breast cancer treatment, evaluation of unexpected enhancing lesions on contrast-enhanced scans obtained for a specific target lesion, and to identify additional cancers during breast cancer staging and preoperative surgical planning (lumpectomy vs mastectomy).

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INTRODUCTION

Magnetic Resonance Spectroscopy (MRS) has emerged as a powerful, non-invasive method for studying tumor biochemistry and has the potential to improve the specificity of contrast-enhanced breast Magnetic Resonance Imaging (MRI). Historically, the phosphorous (^{31}P) nucleus has been most commonly observed in experimental MRS studies of tumors to demonstrate metabolite concentration differences between tumors and normal tissues[1]. Two of these ^{31}P MRS observable compounds, PC and GPC, contain N-trimethyl groups in the choline moiety that are visible in a ^{1}H MR spectrum. The N-trimethyl group contains 9 equivalent protons in choline[2], producing a single resonance at 3.2 ppm arising from all choline-containing compounds. Several *in vivo* and *in vitro* studies have used elevation of this peak to discriminate between normal and neoplastic tissue[2-9]. It is postulated that the concentration of unbound choline-containing compounds increases with cell membrane synthesis and/or number of cells leading to an elevated choline peak at 3.2 ppm. High field strength proton MRS studies of malignant and normal breast tissue extracts suggest that this signal is derived largely from phosphocholine[7-10]. The purpose of our study was to evaluate an *in vivo* MR spectroscopy method that incorporates automated shimming and enhanced *in vivo* water and lipid suppression for differentiating benign and malignant breast lesions by detecting an elevated choline peak in malignancies confirming the presence of choline using *in vitro* MR spectroscopy of breast cell samples and correlating these findings to biopsy results.

The **specific aims** of this project are:

Specific Aim 1: To develop and optimize a new *in vivo* multi-voxel proton MRS technique to measure choline peaks in breast cancer.

Specific Aim 2: To demonstrate that *in vivo* MRS can distinguish cancer from benign breast disease. We hypothesize that in vivo MRS choline measurements will be elevated in breast cancer compared to benign disease. The optimized MRS sequences developed in Year 1 were be used in cancer and benign disease patients in Years 2 and 3. The *in vivo* MRS data is compared to both final histology/cytology and *in vitro* MRS scans of breast cell aspirates.

BODY

Our work was divided into a 3-year study, which was separated into two phases: (Phase 1) technical development and optimization in Year 1 corresponding to Specific Aim 1 and (Phase 2) clinical testing in the Years 2 and 3 corresponding to Specific Aim 2 . This report covers the second year of this three year project.

The first year involved MRS pulse sequence development and optimization for detecting choline while limiting water and lipid signals, and minimizing motion-induced artifacts which can interfere with choline peak detection. Pulse sequence development involved testing and optimization in phantoms and volunteers. We developed a method to analyze breast cells using a high resolution *in vitro* MRS study in a Stanford University chemistry laboratory to validate the *in vivo* MRS data, optimizing our protocol with standards. We hired a nurse coordinator to recruit patients and developed a clinical protocol for MRS scans and patient transportation to the hospital for breast tumor fine-needle aspirations of the breast tumor under direct ultrasound guidance. Given our rapid pulse sequence development, we began early patient recruitment in Year 1 for clinical test scans that were to be done in Years 2 and 3. We recruited patients with suspicious breast lesions and performed a contrast-enhanced diagnostic MRI to localize the lesion. We then used the optimized MRS pulse sequence on the breast mass in the MRI scanner, performed a fine-needle aspiration of the tumor to

obtain breast cells in the hospital, used the optimized *in vitro* MRS study on the cells, then correlated the MRS studies to the cytology/pathology. Last year we reported preliminary data on 5 patients.

This year (Year 2), our most significant technical accomplishments include continued optimization of the MRS techniques for measuring *in vivo* choline levels in the breast. This was accomplished at three levels: pulse sequence development, optimization of RF coil configurations, and improved data reconstruction and quantification algorithms. In particular, a PRESS sequence was modified with the addition of dual-band BASING pulses for added water and lipid suppression. Sharper delineation of the excited volume of tissue was achieved with the addition of up to 6 graphically prescribed highly selective spatial saturation bands. Our phantom and patient data this year showed excellent suppression of water and lipid resonances while passing the choline and/or creatine resonances with no detectable attenuation. Several Rf coils were investigated include volume, surface, and phased array coils. The best results were obtained with a 4-coil phased array breast coil configured to only receive signal from the right or left side respectively depending on the location of the selected lesion. Finally, *in vivo* data was processed using custom software to both detect and correct motion-induced phase variations followed by peak integration.

On the clinical testing side, during year 2 we processed MRS data on scanned patients from Year 1, and scanned an additional 7 patients using the new technical improvements described above. During this second year we also developed volumetric spectroscopic imaging techniques to acquire short-echo-time *in vivo* proton spectra thereby increasing the signal to noise ratio for choline. We decreased the voxel size, and obtained more acquisitions to increase choline detection on the *in vivo* scans. We also scanned both treated and untreated breast cancers with and without intravenous contrast to determine if cancer treatment or if the presence of contrast affected *in vivo* MRS data collection. It has been postulated that Gadolinium-based (Gd) contrast agents may broaden the choline peak resulting in loss of choline signal and inaccurate quantitation of metabolites[11-12]. We acquired our MRS data approximately 25 minutes after initial Gd infusion due to time needed to acquire the diagnostic MRI scans (3DSSMT and spiral dynamic pulse sequences). Using our MRI and MRS techniques, we found that intravenous gadolinium infusion did not significantly affect MRS choline peak detection.

Regarding the *in vitro* MRS scans and technique, this year we focused on increasing the yield of viable breast cell samples on fine-needle aspiration because of our unexpected finding of no choline peak on the *in vitro* MRS scan in a patient with a large treated breast cancer that showed an elevated choline peak on the *in vivo* MRS scan. We postulated that the absence of choline peaks on the *in vitro* MRS scan was due to breast cell samples from a necrotic part of the tumor (since a non-viable treated portion of the tumor is indistinguishable from living cancer on the ultrasound). We increased the sample aspirate by sampling both the center and the periphery of the tumors to increase the probability of obtaining live tumor cells, added a hand-held aspiration device to increase suction during the needle biopsy thereby increasing cell yield, and increasing the total number of excursions through the tumor (15-25 excursions per needle) while not increasing the total number of separate needle sticks into the patient (four needles in total). We obtained the pathology and cytology data on scanned patients and correlated these to the *in vivo* and *in vitro* spectroscopy.

In year 1, we had begun the early accrual of patient subjects and used the optimized MRS sequences in 9 patients with cancer and benign disease. At the time of our last progress report, we had processed and reported data on 5 patients. Our preliminary results had shown that choline measurements were elevated in 3 breast cancers compared to 2 benign breast lesions in both the *in vivo* and *in vitro* MRS scans, with the one exception of a treated cancer patient who showed choline peaks on the *in vivo* scan but no choline peak on the *in vitro* scan of her cell samples.

Of the 16 patients scanned to date, both the *in vivo* and *in vitro* MR spectroscopy data are processed in 11, and data is being processed in 3. Unfortunately the MRS data was irretrievable in 2 patients: 1 was due to a computer malfunction, resulting in computer repairs, and in 1 patient the

choline peak was obscured due to extreme patient motion during the scan. This was the only patient during the study who had so much difficulty with motion during the scan that it resulted in inadequate data collection for MRI and MRS, and overcame our motion-correction sequence. In all other patients the sequence corrected small patient movements, which is essential for obtaining spectroscopic data.

Our preliminary data to date are quite promising, and confirm our expectations that MRS can detect choline in breast cancers. *In vivo* and *in vitro* MRS data in the 11 patients with histological findings are shown in Table 1, Appendix 1. Three patients had benign lesions (post-biopsy scar, fibroadenoma, fibrocystic change/stromal fibrosis); and the remaining 8 patients had cancer (4 invasive ductal cancer, 2 cancers with both invasive ductal and invasive lobular histologies, 1 adenocarcinoma not otherwise specified, 1 invasive ductal carcinoma with mucinous features). Three patients had previously undergone chemotherapy. *In vitro* and *in vivo* results are indicated as a positive or negative for an increase in the N-trimethyl resonance of choline at 3.2 ppm (arising predominantly from the choline of phospholipids). Of the 8 cancers, 6 (75%) showed a choline peak in the *in vivo* MRS scans. The 2 negative studies were seen in patients with cancers containing both invasive ductal and lobular histologies (2 cases). Of 8 cancer patients undergoing *in vitro* MRS, 6 contained elevated choline peaks and 2 had no detectable choline peak (1 invasive ductal/lobular carcinoma, 1 mucinous carcinoma). 2 of the 3 patients with benign disease (1 fibroadenoma, 1 post-biopsy scar) had no choline peak on both the *in vivo* and *in vitro* MRS scans. One patient with fibrocystic change and dense fibrosis had a negative *in vivo* scan but a positive *in vitro* scan.

Our *in vivo* data shows that choline in breast cancer can be detected by *in vivo* MRS scans in most breast cancer patients. We noted that the negative *in vivo* MRS scans occurred in patients with invasive ductal and lobular histology, while patients with invasive ductal cancer always showed choline peaks. The breast cancer histology may influence the ability of MRS to detect tumor choline peaks. Unlike invasive ductal breast cancer, which generally grows as a solid mass of cells, invasive lobular cancer usually infiltrates the breast in single cells, spreading through a large amount of breast tissue and fat in files with little or no discernable mass. We postulate that two negative *in vivo* MRS scans may have occurred because of this phenomenon due to the invasive lobular histology portion of the breast cancers. Baker et al found no choline metabolites, even at 4T in a case of lobular cancer[13].

Our *in vitro* data usually confirmed the existence of elevated choline detected by the *in vivo* MRS scans, and was generally elevated in breast cancers and absent in benign disease. However, breast cancer histology again may have influenced our results on the fine-needle aspirates of the breast cancer cells, resulting in negative *in vitro* MRS scans in 2 cases. The cancer histology is intriguing in that one of the 2 patients with an invasive ductal and lobular cancer showed no choline peak on *in vitro* MRS but the other patient's *in vitro* spectrum exhibited a sharply increased choline peak. It is possible that during the FNA procedure, in the first case the needle sampled an area where there were few cells while in the second case, the needle may have sampled an extremely cellular portion of the tumor, resulting in the elevated choline peak. Similarly, there was a negative *in vitro* spectrum on a patient with an invasive ductal cancer containing mucinous features. In this patient, the needle may have sampled the mucinous portion of the tumor, which contains a gelatinous substance and very few cancer cells, also resulting in a negative *in vitro* MRS scan.

Our results were as expected in patients with benign disease, and were all negative except for a surprising elevated *in vitro* choline peak in a patient with surgically proven benign histology of fibrocystic change and fibrosis. On retrospective review after these results were known, we re-confirmed that the lesion location on MRI, the *in vivo* MRS, the mammogram, the FNA ultrasound images correlated to the location of the fine-needle aspirate and the tissue removed at surgical biopsy. Why did breast cells from surgically proven benign fibrocystic change and fibrosis show an elevated *in vitro* choline peak? It has been reported that surgically induced hypoxia during the FNA

extraction process which may lead to catabolism of Ptdcholine, and since free choline is one of the breakdown products, this could lead to increased choline *in vitro*[3]. Possibly the trauma of the FNA process may have led to the elevated choline peak in this particular patient. Choline can also be detected in healthy breast-feeding patients. In this population, elevated choline peaks might be expected since increased metabolic activity also results in increased choline [8,14], however our patient was not nursing at the time of her scan. Either of these factors or possible natural physiologic fluctuations in choline levels due to hormonal cycles may have contributed to the *in vitro* elevated choline peak in our patient.

In summary, Year 2 of our project has produced additional technical developments of the MRS sequence, resulting in improved spectroscopic images of key metabolites found in breast cancer. We have validated our work with *in vitro* spectra and pathology, and identified possible clinical situations in which MRS may *not* be applicable or may be falsely negative or positive.

In Year 3, we plan to continue technical development of the MRS sequence, and continue clinical testing in patients. Several technical improvements are in the process of being implemented and tested in phantoms. In particular, while the dual-BASING PRESS pulse sequence obtained excellent water suppression along with adequate suppression of the 0.9 and 1.3 ppm lipid peaks, additional lipid resonances, particularly between 2.8-2.3 ppm were not fully suppressed, and, in some cases, obscured the choline peak of interest. Pulse sequence improvements, aimed at improved lipid suppression, including the addition of short TR inversion recovery (STIR) as well as an increased echo time. The transfer of the pulse sequences to high field system (particularly the 3T magnet located a the Lucas Center) should provided improved performance due to both increased SNR and greater spectral peak separation. Spectroscopic data has a higher signal to noise ratio in higher magnetic field strengths, yielding more information on key metabolites. This is especially important in the breast, where fat and water interfere with the choline peak. Finally additional improvements to the spectroscopic imaging acquisitions will provide greatly improved clinical utility by providing full coverage of the breast.

KEY RESEARCH ACCOMPLISHMENTS:

1) MRS pulse sequence development, with additional PRESS sequence modification with the addition of dual-band BASING pulses for added water and lipid suppression
2) The addition of up to 6 graphically prescribed highly selective spatial saturation bands resulting in sharper delineation of the excited volume of tissue
3) Determination of optimal RF coil configurations (4-coil phased array breast coil configured to only receive signal from the right or left side)
4) Improved data reconstruction and quantification algorithms using custom software to both detect and correct motion-induced phase variations followed by peak integration.
5) Processing of *in vivo* MRS and *in vitro* MRS scans using custom software.
6) Correlation and analysis of resulting MRS data with pathology and cytology findings.
7) Continued patient recruitment for *in vivo* MRS scans, fine-needle aspiration and pathology/cytology correlation.

REPORTABLE OUTCOMES:

Peer-reviewed Journals

1. Star-Lack JM, Adalsteinsson E, Gold GE, Ikeda DM, Spielman DM. *Motion correction and lipid suppression for 1H magnetic resonance spectroscopy.* Magn Reson Med, 2000. **43**(3): p. 325-30.3

2. Hunjan SS, Spielman D, Adalsteinsson E, Star-Lack J, Sawyer-Glover A, Ikeda DM. Comparison of in vivo and in vitro 1H magnetic resonance spectroscopy of breast lesions. Submitted to Radiology.

Abstracts

1. Hunjan S, Spielman D, Sawyer-Glover A, Ikeda DM. "Comparison of *In Vivo* and *In Vitro* H MR Spectroscopy of Breast Cancer". International Society for Magnetic Resonance in Medicine, April 2001, Glasgow, Scotland
2. Spielman D, Hunjan S, Sawyer-Glover A, Adalsteinsson E, Ikeda DM. "Proton Spectroscopic Imaging of Breast Cancer". International Society for Magnetic Resonance in Medicine, April 2001, Glasgow, Scotland
3. Rausch-Garrity P, Spielman D, Hunjan S, Sawyer-Glover A, Adalsteinsson E, Ikeda DM. "Dynamic Spiral Imaging K21 Values and Breast Cancer Morphology on High-Resolution 3DSSMT MRI Scans: Correlation with Proton Spectroscopic Imaging of Breast Cancer". International Society for Magnetic Resonance in Medicine, April 2002, Honolulu, Hawaii (to be submitted 12/01).

Degrees supported by this Award

Postdoctoral Research Affiliate: Sandeep Hunjan, PhD

Funding applied for based on work supported by this award

Stanford University Medical Scholars Award. Awardee: medical student Patricia Rausch. Project title: Combining Magnetic Resonance (MR) Spectroscopy and Contrast-enhanced MRI for Breast Cancer Diagnosis 9/1/01-11/30/01

Research opportunities applied for and/or received based on experience/training supported by this award

Susan G. Komen Research Grant. Principal Investigator: Debra M. Ikeda
Project title: Do K21, Parametric Mapping or Tumor Morphology on Contrast-Enhanced Breast Magnetic Resonance Imaging Predict Tumor Response to Chemotherapy? 10/01/01-09/30/03
Total award: $242,479.00

CONCLUSIONS:

We have developed a unique magnetic resonance imaging multi voxel pulse sequence producing spectroscopic images of key metabolites found in breast cancer, and validated our work with *in vitro* spectra and pathology. We have shown that choline peaks are often present in breast cancer, and that our MRS sequence was unaffected by intravenous contrast, increasing its clinical utility as an adjunctive study to clinical breast MRI scans. However, MRS may have a major limitation in that specific tumor histologies that have dispersed cells, such as invasive carcinomas with some invasive lobular carcinoma features may *not* show choline peaks. Technical developments to date have significantly contributed towards the goal of making MR spectroscopic imaging a clinically useful procedure that could be implemented at the time of a contrast-enhanced MRI scan. Important applications for this technique include distinction of a breast cancer recurrence from a

post-biopsy scar within 24 months of initial breast cancer treatment, evaluation of unexpected enhancing lesions on contrast-enhanced scans obtained for a specific target lesion, and to identify additional cancers during breast cancer staging and preoperative surgical planning (lumpectomy vs mastectomy).

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Appendix 1

Table 1. In vivo and in vitro MRS detection of choline compared to pathology in 11 patients*

(a) Pre-Treatment patients

Patient	Pathology	*in vivo*	*in vitro*	Size at MR	Therapy Status
1	IDC with mucinous features	+	_	1.5 cm	No Therapy
2	IDC and ILC	_	_	2.3 cm	No Therapy
3	IDC	+	+	4.5 cm	No Therapy
4	IDC	+	+	8 cm	No Therapy
5	Adenocarcinoma NOS	+	+	2.6 cm	No Therapy

(b) Post-Treatment Patients

Patient	Pathology	*in vivo*	*in vitro*	Size at MR	Therapy Status
6	IDC and ILC	_	+	DIFFUSE	Chemo ONLY
7	IDC	+	+	6 cm	Chemo ONLY
8	IDC	+	+	6 cm	Chemo ONLY

(c) Patients with Benign Disease

Patient	Pathology	*in vivo*	*in vitro*	Size at MR	Therapy Status
9	Scar Tissue	–	–	2 cm	Chemo+Rad
10	Fibro Adenoma	–	–	1.5 cm	No Therapy
11	Fibrocystic Change and Stromal fibrosis	–	+	2 cm	No Therapy

IDC= invasive ductal cancer, ILC=invasive lobular cancer, NOS= not otherwise specified

***3 additional patients pending MRS data analysis, 2 other patients not included due to technical problems.**